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(71) Applicant *(for all designated States except US)*: **GERON CORPORATION** [US/US]; 230 Constitution Drive, Menlo Drive CA 94025 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VILLEPONTEAU, Bryant [US/US]; 4953 Smith Canyon Court, San Diego, CA 92130 (US). HARLEY, Calvin [CA/US]; 1730 University Avenue, Palo Alto, CA 94301 (US).

(74) Agents: SMITH, William, M. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111 (US).

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(57) Abstract

Telomerase reporter constructs are suitable for use in reporting transcriptional activity of a mammanlian telomerase gene transcription regulatory regions. The constructs contain a transcription regulatory region of a mammalian telomerase gene operably linked to a reporter polynucleotide sequence.

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ASSAYS FOR REGULATORS OF MAMMALIAN TELOMERASE EXPRESSION

BACKGROUND OF THE INVENTION

The present invention relates to mammalian telomerase genes and transcriptional regulatory regions thereof. The invention provides methods, cell lines, transgenic animals, and compositions relating to the fields of molecular biology, chemistry, pharmacology, and medical and diagnostic technology.

Telomerase is a ribonucleoprotein enzyme that synthesizes one strand of the telomeric DNA using as a template a sequence contained within the RNA component of the enzyme. See Blackburn (1992) *Annu. Rev. Biochem.* 61:113-129. Mammalian telomerase is composed of one or more protein components and an RNA component. The RNA component comprises a template repeat sequence complementary to the telomere repeat sequence. The RNA component of various mammalian telomerases, including human telomerase ("hTR"), have been reported. See, e.g., Feng et al. (1995) *Science* 269:1267; Villeponteau et al., International application WO 96/01835, published 25 January 1996; Andrews et al., International application WO 96/01614, published January 25, 1996, and Villeponteau et al., United States patent 5,583,016.

Telomeres have an important biological role in maintaining chromosome structure and function. Current evidence is consistent with the idea that a loss of telomeric DNA acts as a trigger of cellular senescence and aging and that regulation of telomerase has important biological implications (see Harley (1991) *Mutation Research* 256:271). Telomerase activity has also been correlated with neoplastic transformation and cancer, wherein cancer cells characteristically exhibit telomerase activity. Most cancer cells or immortalized cell lines express high levels of telomerase activity, while in most normal somatic human cells, telomerase is not detected (Kim et al. (1994) *Science* 266:2011). Expression of antisense RNA complementary to hTR in an immortal human

cancer cell line (HeLa) has been shown to induce cell crisis and cell death after a number of cell doublings (Villeponteau et al., International application WO 96/01835, *supra*).

The transcriptional regulation of telomerase genes and factors or agents which can influence the expression of telomerase genes are not well-defined in the art. The development of transcriptional regulators which would afford a basis to control the expression of specific genes, such as telomerase genes, is a desired goal. Such transcriptional regulators can be pharmaceuticals for treating or preventing telomerase-related pathological conditions.

There is a great need in the art for methods and systems for identifying agents which regulate expression of mammalian telomerase. Significant improvements to and new opportunities for telomerase-mediated therapies and drug development methods could be realized if methods and systems for evaluating transcriptional effects of agents on expression of genes encoding the RNA component and/or encoding the protein components of telomerase were available.

The present invention meets these and other needs and provides such improvements and opportunities. The references discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

SUMMARY OF THE INVENTION

The present invention provides methods, reagents, genetically modified animals and cells, and pharmaceutical compositions relating to the ribonucleoprotein telomerase. Preferably, the telomerase is of mammalian origin; human telomerase is especially preferred. The invention also relates to methods and compositions for identifying agents which modulate transcription, and more particularly which modulate transcription position effect and/or telomerase gene expression. Fig. 2 shows structure of some reporter polynucleotides of the invention.

In one aspect this invention provides a telomerase reporter construct recombinant polynucleotide comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence encoding a reporter polynucleotide heterologous to the transcription regulatory region, wherein the transcription regulatory region comprises nucleotide sequences sufficient for activating

transcription of the reporter polynucleotide and wherein expression of the reporter polynucleotide is detectable. The construct is useful in reporting transcriptional activity of a mammalian telomerase gene transcription regulatory region.

In one embodiment the transcription regulatory region of a mammalian telomerase component gene is a transcription regulatory region of a mammalian telomerase RNA component gene and, more particularly, a transcription regulatory region of a human telomerase RNA component ("hTR") gene. In another embodiment the transcription regulatory region comprises at least a TATA box consensus sequence and a CCAAT box consensus sequence of the hTR gene locus. In another embodiment the transcription regulatory region is hTR-expressing cell specific. In another embodiment the transcription regulatory region comprises a contiguous sequence of at least 1.4 kb upstream, at least 5 kb upstream, at least 10 kb upstream, or at least 20 kb upstream of nucleotide 1459 of SEQ ID NO:1.

In another embodiment the reporter polynucleotide sequence encodes a selectable drug marker, β-galactosidase, fluorescent protein, chloramphenicol acetyltransferase or a sequence that specifically hybridizes to a preselected oligonucleotide probe.

In another aspect this invention provides a recombinant host cell comprising a telomerase reporter construct recombinant polynucleotide of this invention.

In another aspect this invention provides a method for determining whether an agent modulates transcription of a nucleotide sequence operably linked to a transcription regulatory region of a mammalian telomerase gene. The method comprises the steps of (a) incubating, under physiological conditions suitable for transcription, a telomerase reporter construct recombinant polynucleotide of this invention; (b) contacting the construct with the agent; (c) measuring the amount of expression of the reporter polypeptide from the construct; and (d) determining whether the measured amount is different than a control amount of expression from the construct which has not been contacted with the agent. A difference between the measured amount and the control amount indicates that the agent modulates transcription from the transcription regulatory region. In certain embodiments the method is conducted (a) *in vitro*, (b) *in vivo* wherein the step of incubating comprises incubating a recombinant host cell transfected with the telomerase reporter construct and wherein the telomerase regulatory region is telomerase-expressing cell specific to the recombinant host cell or (c) *in vivo* and comprising the use

of a transgenic animal having a genome having the telomerase reporter construct. Expression includes expression, processing, assembly or stability of the reporter polynucleotide sequence.

In another aspect this invention provides an agent that modulates transcription from a transcription regulatory region of a mammalian telomerase gene determined by the above method.

In another aspect, this invention provides a transgenic non-human animal comprising a genomic copy of a telomerase reporter construct. In a variation, the telomerase reporter construct is present in the genome of the animal as a germline copy, or multiple copies, which are non-homologously integrated into one or more chromosomal locus. In a variation, the telomerase reporter construct is present in the genome of the animal as a germline copy, or multiple copies, which are homologously integrated into a chromosomal locus. In a variation, the telomerase reporter construct is present in the genome of a subset of somatic cells in the animal and is substantially not present in germ cells. In a variation, the telomerase reporter construct is present in the genome of cells of one organ, tissue, or cell type of the animal and is substantially absent in other cells of the animal.

In another aspect this invention provides a method for prophylactic or therapeutic treatment of a telomerase-related condition comprising the step of administering to a subject a pharmacologically effective amount of an agent that modulates transcription from a transcription regulatory region of a mammalian telomerase gene determined by the above method. In one embodiment, the telomerase-related condition in cancer and the modulatio is repression of transcription.

In another aspect this invention provides a tagged RNA component construct recombinant polynucleotide comprising a transcription regulatory region operably linked to a nucleotide sequence encoding a tagged telomerase RNA component, wherein the component comprises a mammalian telomerase RNA component sequence and a tag sequence.

In another aspect this invention provides a method for determining whether an agent modulates association between a mammalian telomerase RNA component and a mammalian telomerase protein component. The method comprises the steps of: (a) contacting a tagged telomerase RNA component comprising a mammalian telomerase RNA component sequence and a tag sequence with a mammalian telomerase protein

component and with an agent; (b) measuring the amount of mammalian telomerase protein component bound to the tagged telomerase RNA component, and (c) determining whether the measured amount is different than a control amount of binding between the mammalian telomerase protein component and the tagged telomerase RNA component which has not been contacted with the agent. A difference between the measured amount and the control amount indicates that the agent modulates binding between the mammalian RNA telomerase component and the telomerase protein component.

In another aspect this invention provides a method for generating position effect reporter cells. The method comprises the steps of: (a) introducing into a population of mammalian cells a reporter expression cassette comprising a transcription regulatory region operably linked to a nucleotide sequence encoding a selectable drug marker gene, whereby the reporter expression cassette is integrated or homologously recombined into a chromosome in a plurality of the cells; (b) culturing the population in the presence of an agent that inhibits position effect; (c) exposing the population to a positive selection agent that selects for cells expressing the selectable drug marker, thereby producing a selected population; (d) culturing the selected population in the substantial absence of said agent and (e) exposing the selected population to a negative selection agent which selects for cells which substantially lack expression of the selectable drug marker, thereby generating a doubly-selected subpopulation enriched in position effect reporter cells wherein a reporter cell has an integrated a reporter expression cassette comprising a nucleotide sequence exhibiting position effect.

In another aspect this invention provides a method for determining whether a test agent inhibits chromatin position effect. The method comprises the steps of: (a) providing a population of position effect reporter cells, each cell comprising an integrated reporter expression cassette that comprises a transcription regulatory region operably linked to a nucleotide sequence encoding a reporter polynucleotide, wherein expression of the nucleotide sequence in the population is greater in a cell population exposed to an agent known to inhibit position effect than in a population not exposed to the agent; (b) culturing the population without an agent known to inhibit position effect; (c) contacting the cell with the test agent; and (d) detecting increased expression of expression of the reporter polynucleotide. Detecting increased expression provides a determination that the test agent inhibits chromatin position effect.

In another aspect this invention provides a method for inhibiting the growth of a cell that expresses telomerase comprising transfecting the cell with an expression cassette comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence coding for the expression of a product that inhibits growth of the cell. In one embodiment, the agent is a polynucleotide, such as an antisense RNA transcribed from an antisense cDNA expression library member transfected into a position effect reporter cell.

In another aspect, this invention provides therapeutic agents which inhibit neoplasia or apoptosis by modulating telomerase function by inhibiting or augmenting formation of telomerase RNA component or protein component by modulating transcription of one or more of the telomerase genes; such agents can be used as pharmaceuticals. Such pharmaceuticals will be used to treat a variety of human and veterinary diseases, such as: neoplasia, hyperplasia, neurodegenerative diseases, aging, AIDS, fungal infection, and the like.

In one embodiment, the invention provides methods for identifying, from a bank or library of agents, candidate telomerase gene transcription modulating agents which modulate telomerase gene transcription and/or transcription of genes that are under position effect influence of a telomere.

In another embodiment, candidate telomerase gene transcription modulating agents are identified by their ability to produce a statistically significant increase or decrease in transcription initiation rate and/or RNA transcript abundance of a reporter polynucleotide sequence operably linked to a mammalian telomerase transcription regulatory region of a telomerase protein component gene or a telomerase RNA component gene.

In another embodiment, candidate telomerase transcription modulating agents are identified by their ability to produce a statistically significant reduction or increase in transcription of a reporter polynucleotide sequence (e.g., β-galactosidase gene, luciferase gene, HPRT gene, etc.) operably linked to a transcriptional regulatory sequence of a mammalian telomerase RNA component gene, preferably a human telomerase RNA component gene, in a metabolically active mammalian cell. In a variation, an endogenous telomerase RNA component gene in a mammalian cell is targeted with a homologous targeting construct to place a reporter polynucleotide sequence in operable linkage to the upstream transcription regulatory sequence (e.g.,

promoter) of the endogenous telomerase RNA component gene in the chromosomal locus of the endogenous gene. In an alternative variation, an exogenous polynucleotide comprising a reporter polynucleotide is operably linked to a mammalian telomerase RNA component gene transcription regulatory region (e.g., promoter and upstream transcription factor binding sites); the exogenous polynucleotide is transferred into a mammalian cell wherein it may integrate non-homologously into a chromosomal location and/or is maintained or replicated as an episomal polynucleotide. Transcription of the reporter polynucleotide sequence can be determined by any suitable method, including but not limited to determining a phenotypic characteristic conferred on a cell by a protein encoded by the reporter polynucleotide sequence, detection by hybridization with a complementary probe polynucleotide, detection by PCR amplification, or the like. Agents which produce a statistically significant transcriptional modulation of the reporter polynucleotide in cells treated with the agent are thereby identified as candidate mammalian telomerase transcription modulating agents.

In another aspect, the invention provides chimeric alleles of mammalian telomerase RNA component genes, wherein a heterologous transcription regulatory region drives expression of an operably-linked mammalian telomerase RNA component gene to functionally express the telomerase RNA component gene under control of a heterologous promoter, which may be constitutive, inducible, tissue-specific, and/or derived from the same or different species of animal as the RNA component encoding gene. In an alternative embodiment, the chimeric gene comprises a telomerase gene transcription regulatory region that drives expression of an operably-linked reporter gene to functionally express the reporter gene under control of the telomerase gene promoter. Transgenic animals harboring a germline copy of a transgene comprising such chimeric genes can be made.

In a variation, a chimeric gene comprising a telomerase transcription regulatory region operably linked to a reporter gene is constructed by homologous gene targeting to operably link said reporter gene to the telomerase gene promoter in the telomerase gene locus. Transgenic animals of these types are used as commercial reagents for toxicology screening, for sale to pharmaceutical research laboratories to identify or investigate telomerase-modulating agents, as pets, and as agricultural livestock among other uses.

In another aspect, this invention provides methods of inhibiting the growth of a cell that expresses telomerase. Such methods are useful, for example, in the treatment of malignant cells that express telomerase, in sterilization by inhibiting the growth of germ cells or in eliminating telomerase-expressing cells from cell cultures. The methods involve transfecting the cell with an expression cassette comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence coding for the expression of a product lethal to the cell. The expression cassette can be part of a vector used in gene therapy, such as an adenoviral or retroviral vector. The lethal product can be directly lethal or lethal after administration of a co-factor. Examples of genes encoding lethal products are ion channel toxins or gpt, which becomes lethal upon administration of 6-thio-Guanine. It may be useful to titrate the agent or modulate the general strength of the overall transcription unit to ensure that leaky expression from the transcription regulatory region does not kill normal cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows schematically an example of a telomerase reporter construct exemplified as a reporter polynucleotide sequence operably linked to a transcription regulatory region of a mammalian telomerase gene in Panel (A), and with transcriptional elements and specific components of a particular construct in Panel (B).

Fig. 2 shows a method of this invention for determining whether an agent inhibits binding of a mammalian RNA component of telomerase from binding with a protein component of telomerase. In the embodiment shown, a tagged RNA component construct contains a heterologous promoter ("hp") operably linked with a sequence encoding a tag ("tag") and a sequence coding for the expression of hTR ("hTR"). Expression of this sequence provides a tagged hTR polynucleotide. The cell expresses both the tagged hTR polynucleotide and telomerase protein component ("TPC"). The tagged hTR associates with TPC. Agents are tested for the ability to inhibit this association. The tagged construct and any associated TPC is isolated by, e.g., affinity methods. In the embodiment shown, the tag includes a *tar* sequence. The affinity reagent includes a *tat* moiety that binds to *tar* and an attached ligand ("L"). The complex is isolated using a binding substance ("BS") that recognizes the ligand.

Fig. 3 shows a method of this invention for inhibiting growth (in this case killing) cells that express telomerase. A cancer cell contains transcription factors ("TF") that activate telomerase transcription regulatory elements ("TTR") that result in expression of human RNA component of telomerase ("hTR") that assembles into telomerase ("T"). Exposure to a vector ("V") transfects the cell with an expression cassette that includes a TTR operably linked to a lethal gene ("L"). The transcription factors activate transcription from the TTR to cause expression of the lethal gene product, which kills the cell.

DESCRIPTION OF THE PREFERRED EMBODIMENT

I. *DEFINITIONS*

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., *Dictionary of Microbiology and Molecular Biology* (2d ed. 1994); *The Cambridge Dictionary of Science and Technology* (Walker ed., 1988); and Hale & Marham, *The Harper Collins Dictionary of Biology* (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

"Polynucleotide" refers to a polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs, such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide nucleic acids ("PNAs"), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. "Nucleic acid" typically refers to large polynucleotides. "Oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences"; sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant polypeptide." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well. Appropriate unicellular hosts include any of those routinely used in expressing eukaryotic or mammalian polynucleotides, including, for example, prokaryotes, such as *E. coli*; and eukaryotes, including for example, fungi, such as yeast; and mammalian cells, including insect cells (e.g., Sf9) and animal cells such as CHO, R1.1, B-W, L-M, African Green Monkey Kidney cells (e.g. COS 1, COS 7, BSC 1, BSC 40 and BMT 10) and cultured human cells.

"Telomerase" or "telomerase ribonucleoprotein complex" refers to a ribonucleoprotein enzyme of eukaryotic origin identifiable by its ability to polymerize a DNA sequence of a eukaryotic telomere. Telomerase is further characterized by an RNA component having sequences complementary to at least part of the telomeric repeat of the source species and by one or more telomerase protein components. As used herein, "animal telomerase," "mammalian telomerase" and "human telomerase" refer to telomerases that can be found naturally in various multicellular animal, mammalian or human cells, respectively, or having polypeptide components with the same amino acid sequences, and RNA components with the same nucleotide sequences. Human

telomerase contains the RNA component, "hTR." The term "telomerase" includes all allelic forms of telomerase, including wild-type and mutant forms.

"Telomerase RNA component" refers to an RNA component of telomerase core enzyme that comprises nucleotide sequences complementary to at least part of a telomeric repeat sequence, and that functions as a template in telomeric DNA synthesis. This includes, without limitation, a primate telomerase RNA component such as a human or monkey telomerase RNA component or the cognate gene in heterologous species (e.g., mouse or rat). Some telomerase RNA component alleles have sequence variations as compared to known naturally-occurring telomerase RNA component genes. A preferred RNA component is a full length human telomerase RNA component (hTR). (See SEQ ID NO:2; Feng et al. (1995) *Science* 269:1267; Villleponteau et al., International application WO 96/01835, published 25 January 1996; Andrews et al., International application WO 96/01614, published January 25, 1996, and Villeponteau et al., United States patent 5,583,016.)

"Telomerase protein component" refers to a protein component of the telomerase core enzyme.

"Telomerase core enzyme" refers to the assembled collection of telomerase components, both the RNA and protein components, sufficient for telomerase activity *in vitro*.

"Telomerase associated protein" refers to a protein that binds to the telomerase core enzyme but that is not necessary for telomerase activity *in vitro*.

"Telomerase activity factor" refers to a protein which, when included with telomerase core enzyme, improves telomerase activity *in vitro*.

"Telomerase related protein" refers, collectively, to telomerase protein components, telomerase associated proteins and telomerase activity factors.

"Telomerase-dependent gene" refers to a gene which manifests an altered rate of transcription, either increased or decreased, from a major or minor transcriptional start site for said gene, wherein such alteration in transcriptional rate correlates with the expression of a telomerase gene (e.g., hTR gene).

"Telomerase activity" refers to the synthesis of telomeric DNA by telomerase. One assay method for detecting telomerase activity is the TRAP assay. See Harley et al., International Application WO 95/13381 and United States patent 5,629,154. This assay measures the amount of radioactive nucleotides incorporated into

elongation products, polynucleotides, formed by nucleotide addition to a telomerase substrate or primer. The radioactivity incorporated can be measured as a function of the intensity of a band on a PhosphorImager™ screen exposed to a gel on which the radioactive products are separated. A test experiment and a control experiment can be compared by visually using the PhosphorImager™ screens. See also the commercially available TRAP-eze™ telomerase assay kit (Oncor); and Morin, *Cell* 59: 521-529 (1989).

"Mammalian telomerase gene locus" refers to a genetic locus at which is located a mammalian telomerase gene and cis-acting expression control sequences operably linked to the mammalian telomerase gene.

"Expression control sequence" refers to a nucleotide sequence in a polynucleotide that regulates the expression (transcription and/or translation) of a nucleotide sequence operatively linked to it. "Operatively linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Expression control sequences can include, for example and without limitation, sequences of promoters (e.g., inducible, repressible or constitutive), enhancers, transcription terminators, a start codon (i.e., ATG), splicing signals for introns, and stop codons.

"Transcription regulatory region" refers to an expression control sequence comprising a functional promoter and any associated upstream or downstream transcription elements (e.g., a CCAAT box consensus sequence, a TATA ("A/T") box consensus sequence, an enhancer, an SP1 site, etc.) that are essential (i.e, that are, together, sufficient) for activating detectable transcription of a polynucleotide sequence that is operably linked to the transcription regulatory region.

"Transcription regulatory region of a mammalian telomerase gene" refers to a transcription regulatory region derived from a mammalian telomerase gene locus. In certain embodiments the mammalian telomerase transcription regulatory region is "telomerase-expressing cell specific," that is, it allows transcription in cells of mammalian origin that express the telomerase gene, but substantially does not allow transcription in cells that do not express the telomerase gene.

"hTR gene promoter region" refers to sequences encoding transcription regulatory elements located upstream of the hTR gene, i.e., upstream of nucleotide 1459

of SEQ ID NO:1, e.g., within about 1.4 kb of the hTR gene, such as nucleotides 1-1458 of SEQ ID NO:1.

"Minimal promoter" refers to a transcription regulatory region comprising nucleotide sequences of the TATA (A/T) box consensus sequence and the CCAAT box consensus sequences, and sufficient to drive transcription of a nucleotide sequence operatively linked thereto.

"Transcriptional modulation" refers to the capacity to either enhance transcription or inhibit transcription of a structural sequence or reporter sequence linked in cis. Such enhancement or inhibition may be contingent on the occurrence of a specific event, such as stimulation with an inducer and/or may only be manifest in certain cell types. The altered ability to modulate transcriptional enhancement or inhibition may affect the inducible transcription of a gene, or may effect the basal level transcription of a gene, or both.

"Transcriptional enhancement" refers to the functional property of producing an increase in the rate of transcription of linked sequences that contain a functional promoter.

"Transcriptional regulatory element" refers to a DNA sequence which activates transcription alone or in combination with one or more other DNA sequences. A transcriptional regulatory element can, for example, comprise a promoter and/or enhancer.

"Transcription factor recognition site" and "transcription factor binding site" refer to a polynucleotide sequence(s) or sequence motif(s) which is identified as being a site for the sequence-specific interaction of one or more transcription factors, frequently taking the form of direct protein-DNA binding. Typically, transcription factor binding sites can be identified by DNA foot-printing, gel mobility shift assays, and the like, and/or can be predicted on the basis of known consensus sequence motifs, or by other methods known to those of skill in the art. For example and not to limit the invention, eukaryotic transcription factors include, but are not limited to: NFAT, AP1, AP-2, Sp1, OCT-1, OCT-2, OAP, NFκB, CREB, CTF, TFIIA, TFIIB, TFIID, Pit-1, C/EBP, SRF (Mitchell PJ and Tijan R (1989) *Science* 245: 371). For purposes of the invention, steroid receptors, RNA polymerases and associated factors, and other proteins that interact with DNA in a sequence-specific manner and exert transcriptional regulatory effects are considered transcription factors.

"Transcriptional unit" and "transcriptional complex" refer to a polynucleotide sequence that comprises a structural gene (exons), a cis-acting linked promoter and other cis-acting sequences necessary for efficient transcription of the structural sequences, distal regulatory elements necessary for appropriate tissue-specific and developmental transcription of the structural sequences, and additional cis sequences important for efficient transcription and translation (e.g., polyadenylation site, mRNA stability controlling sequences).

A "reporter polynucleotide" is a polynucleotide whose expression can be detected (e.g., detected directly or through expression of a phenotype, screened for and/or selected for). Preferably, a detection assay can quantitate the relative level of expression of a reporter polynucleotide. For example, a reporter polynucleotide can encode a detectable reporter protein (e.g., luciferase, β-galactosidase, chloramphenicol acetyltransferase, HPRT, fluorescent protein, thymidine kinase, neoR, and the like). A sequence encoding a reporter polynucleotide can comprise an inducible or constitutive enhancer-promoter directing transcription of a sequence.

Such a reporter polynucleotide may be transferred to a responsive or competent cell line for use as a reporter host cell to screen a panel of agents for the ability to produce transcriptional modulation. Agents that enhance transcription of the cis-linked reporter gene are identified as putative positive regulators of transcription. Numerous other specific examples of transcription regulatory elements, such as specific enhancers and silencers, are known to those of skill in the art and may be selected for use in the methods and polynucleotide constructs of the invention on the basis of the practitioner's desired application. Literature sources and published patent documents, as well as GenBank™ and other sequence information data sources can be consulted by those of skill in the art in selecting suitable transcription regulatory elements for use in the invention. Where necessary, a transcription regulatory element may be constructed by synthesis (and ligation, if necessary) of oligonucleotides made on the basis of available sequence information (e.g., GenBank sequences for a CD4 enhancer or a SV40 early promoter). The altered ability to modulate transcriptional enhancement or inhibition may affect the inducible transcription of a gene, the basal level transcription of a gene, or both. Agents that disrupt, for example, binding of silencer proteins to silencer transcription regulatory elements typically produce an increase in basal and/or induced transcription rate of a cis-linked gene.

A "selectable drug marker" is a gene whose expression in a cell confers on the cell resistance or sensitivity to a drug.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient *cis*-acting elements for expression; other elements for expression can be supplied by the host cell or *in vitro* expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide.

"Reporter expression cassette" refers to a recombinant polynucleotide comprising a promoter sequence and, optionally, an enhancer and/or silencer element(s), operably linked to a structural sequence, such as a cDNA sequence or genomic DNA sequence encoding a reporter protein (e.g., luciferase, β-galactosidase, chloramphenicol acetyltransferase), such that the reporter gene sequence is under the transcriptional influence of a cis-acting transcription factor binding site and/or recognition site. In some embodiments, an expression cassette may also include polyadenylation site sequences to ensure polyadenylation of transcripts. When a reporter expression cassette is transferred into a suitable host cell, the structural sequence is transcribed from the expression cassette promoter, and a translatable message is usually generated, either directly or following appropriate RNA splicing.

"Reporter host cell" refers to a eukaryotic cell, preferably a mammalian cell, which harbors a reporter expression cassette. Preferably, the reporter expression cassette polynucleotide is stably integrated into a host cell chromosomal location, either by non-homologous integration or by homologous sequence targeting, although transient transfection methods may be employed.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided

in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

"Allelic variant" refers to any of two or more polymorphic forms of a gene occupying the same genetic locus. Allelic variations arise naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. "Allelic variant" also refers to polymorphisms in non-coding sequences at a genetic locus and cDNAs derived from mRNA transcripts of genetic allelic variants, as well as the proteins encoded by them.

"Hybridizing specifically to" or "specific hybridization" or "selectively hybridize to," refers to the binding, duplexing, or hybridizing of a polynucleotide preferentially to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA.

"Stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of polynucleotide hybridization experiments such as Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of polynucleotides is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe.

An example of stringent hybridization conditions for hybridization of complementary polynucleotides which have more than 100 complementary residues on a

filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42° C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72° C for about 15 minutes. An example of stringent wash conditions is a 0.2X SSC wash at 65° C for 15 minutes (see Sambrook et al. for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45° C for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6x SSC at 40° C for 15 minutes. In general, a signal-to-noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

A first sequence is an "antisense sequence" with respect to a second sequence if a polynucleotide whose sequence is the first sequence specifically hybridizes with a polynucleotide whose sequence is the second sequence.

"Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Probe" refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes can

be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Detecting" refers to determining the presence, absence, or amount of an analyte in a sample, and can include quantifying the amount of the analyte in a sample or per cell in a sample.

"Modulating" refers to detectably increasing or decreasing an activity or association.

The terms "label" or "labeled" refer to incorporation of a detectable marker, e.g., a radiolabeled nucleotide or amino acid, or a recoverable label (e.g. biotinyl moieties that can be recovered by avidin or streptavidin). Recoverable labels can include covalently linked polynucleobase sequences that can be recovered by hybridization to a complementary sequence polynucleotide. Various methods of labeling polypeptides and polynucleotides are known in the art and may be used. Examples of labels include, but are not limited to, the following: radioisotopes (e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I, ^{131}I), fluorescent or phosphorescent labels (e.g., FITC, rhodamine, lanthanide phosphors, fluorescent proteins), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., digoxigenin, leucine zipper pair sequences, binding sites for antibodies, transcriptional activator polypeptide, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths, e.g., to reduce potential steric hindrance.

"Linker" refers to a molecule that joins two other molecules, either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., a polynucleotide that hybridizes to one complementary sequence at the 5' end and to another complementary sequence at the 3' end, thus joining two non-complementary sequences.

"Amplification" refers to any means by which a polynucleotide sequence is copied and thus expanded into a larger number of polynucleotides, e.g., by reverse transcription, polymerase chain reaction, and ligase chain reaction.

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. The term

"protein" typically refers to large polypeptides. The term "peptide" typically refers to short polypeptides.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

"Complementary" refers to the topological compatibility or matching together of interacting surfaces of two polynucleotides. Thus, the two molecules can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other. A first polynucleotide is complementary to a second polynucleotide if the nucleotide sequence of the first polynucleotide is identical to the nucleotide sequence of the polynucleotide binding partner of the second polynucleotide. Thus, the polynucleotide whose sequence 5'-TATAC-3' is complementary to a polynucleotide whose sequence is 5'-GTATA-3'.

"Antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and F(ab)'$_2$ fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies, as well as humanized antibodies.

An antibody "specifically binds to" or "is specifically immunoreactive with" a protein when the antibody functions in a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically

immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

"Immunoassay" refers to an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The term "naturally-occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "cognate" refers to a gene sequence that is evolutionarily and functionally related between species. For example but not limitation, in the human genome, the human CD4 gene is the cognate gene to the mouse CD4 gene, since the sequences and structures of these two genes indicate that they are highly homologous and both genes encode a protein which functions in signaling T cell activation through MHC class II-restricted antigen recognition.

The term "agent" refers to a chemical compound, a mixture of chemical compounds, an array of spatially localized compounds (e.g., a VLSIPS peptide array, polynucleotide array, and/or combinatorial small molecule array), a biological macromolecule, a bacteriophage peptide display library, a bacteriophage antibody (e.g., scFv) display library, a polysome peptide display library, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents are evaluated for potential activity as transcription modulatory agents (e.g., telomerase gene antagonists or agonists, antineoplastic agents, cytotoxic agents, cell proliferation-promoting agents, and the like) by inclusion in screening assays described herein or the like. Agents are evaluated for potential activity as specific telomerase gene transcription modulators (i.e., an agent which selectively modulates transcription of a telomerase gene but which does not substantially interfere with cell viability) by inclusion in screening assays described hereinbelow.

"Physiological conditions" refers to temperature, pH, ionic strength, viscosity, and like biochemical parameters that are compatible with a viable organism, and/or that typically exist intracellularly in a viable cultured mammalian cell, particularly

conditions existing in the nucleus of said mammalian cell. For example, the intranuclear or cytoplasmic conditions in a mammalian cell grown under typical laboratory culture conditions are physiological conditions. Suitable *in vitro* reaction conditions for *in vitro* transcription cocktails are generally physiological conditions, and may be exemplified by a variety of art-known nuclear extracts. In general, *in vitro* physiological conditions can comprise 50-200 mM NaCl or KCl, pH 6.5-8.5, 20-45°C and 0.001-10 mM divalent cation (e.g., Mg^{++}, Ca^{++}); preferably about 150 mM NaCl or KCl, pH 7.2-7.6, 5 mM divalent cation, and often include 0.01-1.0 percent nonspecific protein (e.g., BSA). A non-ionic detergent (Tween, NP-40, Triton X-100) can often be present, usually at about 0.001 to 2%, typically 0.05-0.2% (v/v). Particular aqueous conditions may be selected by the practitioner according to conventional methods. For general guidance, the following buffered aqueous conditions may be applicable: 0-250 mM NaCl, 5-50 mM Tris HCl, pH 5-8, with optional addition of divalent cation(s), metal chelators, nonionic detergents, membrane fractions, anti-foam agents, and/or scintillants.

The term "statistically significant" means a result (i.e., an assay readout) that generally is at least two standard deviations above or below the mean of at least three separate determinations of a control assay readout and/or that is statistically significant as determined by Student's t-test or other art-accepted measure of statistical significance.

"Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a mammal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and a pharmaceutically acceptable carrier. "Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result. "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in *Remington's Pharmaceutical Sciences*, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (e.g., oral) or parenteral (e.g., subcutaneous, intramuscular, or intravenous intraperitoneal injection; or topical, transdermal, or transmucosal administration).

"Telomerase-related condition" refers to a condition in a subject maintained by telomerase activity within cells of the individual. Telomerase-related conditions include, e.g., cancer (telomerase-activity in malignant cells), fertility (telomerase activity in germ-line cells) and hematopoiesis (telomerase activity in hematopoietic stem cells).

The term "anti-neoplastic agent" refers to agents that have the functional property of inhibiting the development or progression of a neoplasm in a mammal, e.g., a human, and may also refer to the inhibition of metastasis or metastatic potential.

A "subject" of diagnosis or treatment is a mammal, including a human. Non-human animals subject to treatment include, for example, domesticated animals.

A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs of pathology for the purpose of diminishing or eliminating those signs.

The term "endogenous DNA sequence" refers to naturally-occurring polynucleotide sequences contained in a eukaryotic cell. Such sequences include, for example, chromosomal sequences (e.g., structural genes, promoters, enhancers, recombinatorial hot-spots, repeat sequences, integrated proviral sequences).

A "predetermined sequence" is a sequence which may be selected at the discretion of the practitioner on the basis of known or predicted sequence information. An "exogenous polynucleotide" is a polynucleotide which is transferred into a eukaryotic cell.

The term "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual macromolecular species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 to 90 percent of all macromolecular species present in the composition. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single

macromolecular species. Solvent species, small molecules (less than 3000 Daltons), and elemental ion species are not considered macromolecular species.

II. *GENERAL PROTOCOLS*

The nomenclature used hereafter and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described herein often involve well known and commonly employed procedures in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, and microbial culture and transformation (e.g., electroporation, lipofection). The techniques and procedures are generally performed according to conventional methods in the art and various general references (see, generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Oligonucleotides can be synthesized on an Applied BioSystems or other commercially available oligonucleotide synthesizer according to specifications provided by the manufacturer.

Chimeric targeted mice are derived according to Hogan, et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988) and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed., IRL Press, Washington, D.C., (1987).

Embryonic stem cells are manipulated according to published procedures (*Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed., IRL Press, Washington, D.C. (1987); Zjilstra et al., *Nature* 342:435-438 (1989); and Schwartzberg et al., *Science* 246:799-803 (1989)).

III. *TELOMERASE REPORTER CONSTRUCTS*

A. Introduction

This invention provides telomerase reporter constructs for monitoring transcription of genes under the transcriptional control of a telomerase gene promoter. Telomerase reporter constructs are recombinant polynucleotides that comprise a transcription regulation region of a mammalian telomerase gene operatively linked to a nucleotide sequence encoding a reporter polynucleotide. Such constructs are useful, among other things, to identify conditions under which a telomerase promoter is active in

a cell, and in assays to identify compounds that modulate activity of a telomerase promoter. This includes mimicking conditions of naturally occurring telomerase promoters, e.g., in cancer cells.

A telomerase reporter construct can include additional polynucleotide sequences, such as: origins of replication for prokaryotic and/or eukaryotic host cells, sequences for gene targeting or targeted integration by homologous recombination, polyadenylation sequences, splicing sequences, heterologous promoters, predetermined sequence elements, and the like.

B. *Telomerase Gene Transcription Regulatory Regions*

A transcription regulatory region of a telomerase gene used in the recombinant constructs of this invention normally includes at least a minimal promoter capable of driving transcription of the reporter polynucleotide. Generally, this is the native promoter(s) associated with the naturally occurring mammalian telomerase gene, in particular, a telomerase RNA component gene. The regions can further comprise one or more transcription factor recognition sites located within about 20 kilobases of a promoter in the naturally-occurring telomerase gene in the mammalian genome. Often, a transcription regulatory region comprises a substantially contiguous segment of DNA spanning from about 10 kilobases upstream, typically 5 kilobases, frequently at least 1.4 kb upstream of the transcription start site (e.g., for the hTR promoter) and continuing downstream to or through the transcription start site.

DNA sequences within or flanking a mammalian telomerase gene which is preferentially expressed in telomerase-expressing cells contain DNA sequence motifs which function to enhance or drive transcription of the cis-linked gene in cells. These sequences are termed telomerase gene transcriptional regulatory sequences. For many intended purposes, the hTR gene is the preferred suitable source for obtaining telomerase-specific transcription regulatory sequences. Such sequences are isolated and evaluated for their capacity to enhance or drive transcription of an operably linked reporter gene (e.g., CAT) in telomerase-expressing cells and substantially not in other cell types. Minimal functional sequences are defined by deletion analysis and/or linker-scanning mutagenesis and the like, followed by assay of transcriptional activity demonstrating transcription in transfected telomerase-expressing cells but not in other cell types which have also been transfected with minimal reporter constructs.

Often, the transcription regulatory region of the telomerase gene is a predetermined cis-acting mammalian telomerase transcription regulatory sequence that comprises a predetermined polynucleotide sequence, such as an identified promoter sequence, a TATA box, a CCAAT box, a PSE consensus sequence, a recognition site sequence for AP-1, AP-2, Sp1, NFAT, OCT-1, OCT-2, OAP, NFκB, CREB, CTF, TFIIA, TFIIB, TFIID, Pit-1, C/EBP, SRF, or other transcription factor having a known binding site sequence, or the like. Typically, the predetermined cis-acting mammalian telomerase transcription regulatory region comprises subregions which are identifiable by foot-printing patterns produced by proteins present in a nuclear extract of mammalian cells expressing said telomerase gene and/or in cells in which said telomerase gene is substantially not transcribed.

In the recombinant constructs of the invention, such as transgenes, the transcriptional regulatory region is at least the minimal sequence(s) required for efficient cell-type specific expression, which generally is at least a promoter and at least about 0.2 kilobase (kb) upstream of the promoter, preferably at least about 1 to 3 kb upstream of the promoter, more preferably at least about 5 kb upstream of the promoter, and frequently at least about 8 kb or more upstream of the promoter. In certain embodiments the transcription regulatory region of the hTR gene comprises no more than 5 kb, 10 kb or 20 kb upstream of nucleotide 1 of hTR (SEQ ID NO:1).

Frequently, sequences downstream of the promoter, especially intronic sequences, are included in the transgene constructs (Brinster et al. (1988) *Proc. Natl. Acad. Sci.* (U.S.A.) 85: 836).

Usually the sequences upstream of the promoter are used contiguously, although various deletions and rearrangements can be employed. Some desired regulatory elements (e.g., enhancers, silencers) may be relatively position-insensitive, so that the regulatory element will function correctly even if positioned differently in a transgene than in the corresponding germline gene. For example, an enhancer may be located at a different distance from a promoter, in a different orientation, and/or in a different linear order. For example, an enhancer that is located 3' to a promoter in germline configuration might be located 5' to the promoter in a transgene. Where convenient, it is preferred that a contiguous segment of genomic DNA sequence spanning the telomerase gene and containing as much upstream flanking sequence as convenient (typically about 1-10 kb) be used in the telomerase transcription reporter polynucleotide,

transgene or targeting construct, with the reporter gene inserted so as to replace or displace at least the first intron of the gene and to be operably linked to the promoter(s).

A telomerase gene transcriptional regulatory element can comprise a promoter and/or enhancer. For example, a telomerase gene enhancer is identified by deletion analysis of the upstream region between -10 kb and -0.01 kb, which typically can be isolated from the human genome as a restriction fragment. Such an enhancer is termed an "upstream telomerase enhancer." Optionally, the naturally-occurring telomerase promoter of the hTR gene can be included in operable linkage with the upstream enhancer. Alternatively, a heterologous promoter can be operably linked to the upstream enhancer and used to drive expression of an operably linked structural gene sequence (e.g., a toxin gene, reporter gene, or other encoding sequence). Various deletions and point mutations can be made to the upstream sequences of the hTR gene, and each variant evaluated for the ability to drive or enhance transcription of a reporter gene (e.g., CAT) in telomerase-expressing cells (e.g., HT1080 cells) and in cells substantially lacking telomerase expression (e.g., IMR90).

It is further recognized that a telomerase gene may comprise multiple promoters, which may individually be cell type-specific, and it is necessary to operably link the reporter gene to at least one promoter (or other transcriptional element) which confers transcription in telomerase expressing cells (especially neoplastic cells). Transcriptional elements which confer transcription in telomerase non-expressing cells and which are not necessary for efficient transcription in telomerase-expressing cells may be advantageously deleted from the telomerase transcription reporter polynucleotide, transgene or targeting construct to provide additional cell-type specificity.

If the transcription regulatory sequence(s) selected are relatively inefficient in transcribing the reporter gene, it may be desirable to incorporate multiple copies of a telomerase transcription reporter polynucleotide, transgene, or targeting construct to compensate with an enhanced gene dosage.

C. *Human Telomerase RNA Component Gene Locus*

In one embodiment the mammalian telomerase gene locus is the hTR locus. Portions of the hTR gene locus have been cloned and placed on deposit.

A lambda clone designated 28-1 contains an ~15 kb insert containing human telomerase RNA component gene sequences. Clone 28-1 was deposited with the American Type Culture Collection pursuant to the Budapest Treaty and granted accession number ATCC 75925. An ~2.4 kb SauIIIA1-HindIII fragment containing the hTR sequences as well as transcription regulatory sequences of the ~15 kb insert was sequenced. The sequence is presented below as SEQ ID NO:1:

```
                                                    50
GATCAGTTAGAAAGTTACTAGTCCACATATAAAGTGCCAAGTCTTGTACT
                                                   100
CAAGATTATAAGCAATAGGAATTTAAAAAAAGAAATTATGAAAACTGACA
                                                   150
AGATTTAGTGCCTACTTAGATATGAAGGGGAAAGAAGGGTTTGAGATAAT
                                                   200
GTGGGATGCTAAGAGAATGGTGGTAGTGTTGACATATAACTCAAAGCATT
                                                   250
TAGCATCTACTCTATGTAAGGTACTGTGCTAAGTGCAATAGTGCTAAAAA
                                                   300
CAGGAGTCAGATTCTGTCCGTAAAAAACTTTACAACCTGGCAGATGCTAT
                                                   350
GAAAGAAAAAGGGGATGGGAGAGAGAGAAGGAGGGAGAGAGATGGAGAGG
                                                   400
GAGATATTTTACTTTTCTTTCAGATCGAGGACCGACAGCGACAACTCCAC
                                                   450
GGAGTTTATCTAACTGAATACGAGTAAAACTTTTAAGATCATCCTGTCAT
                                                   500
TTATATGTAAAACTGCACTATACTGGCCATTATAAAAATTCGCGGCCGGG
                                                   550
TGCGGTGGCTCATACCTGTAATCCCAGCACTTTGGGAGGCCGAAGCGGGT
                                                   600
GGATCACTTGAGCCCTGGCGTTCGAGACCAGCCTGGGCAACATGGTGAAA
                                                   650
CCCCCGTCTCTACTAAAAACACAAAAACTAGCTGGGCGTGGTGGCAGGCG
                                                   700
CCTGTAATCCCAGCTACTCAGGAGGCTGAGACACGAGAATCGCTTGAACC
                                                   750
CGGGAGCAGAGGTTGCAGTGAGCCGAGATCACGCCACTAGACTCCATCCA
                                                   800
GCCTGGGCGAAAGAGCAAGACTCCGTCTCAAAAAAAAAAATCGTTACAAT
                                                   850
TTATGGTGGATTACTCCCCTCTTTTTACCTCATCAAGACACAGCACTACT
                                                   900
TTAAAGCAAAGTCAATGATTGAAACGCCTTTCTTTCCTAATAAAAGGGAG
                                                   950
ATTCAGTCCTTAAGATTAATAATGTAGTAGTTACACTTGATTAAAGCCAT
                                                  1000
CCTCTGCTCAAGGAGAGGCTGGAGAAGGCATTCTAAGGAGAAGGGGGCAG
                                                  1050
GGTAGGAACTCGGACGCATCCCACTGAGCCGAGACAAGATTCTGCTGTAG
                                                  1100
TCAGTGCTGCCTGGGAATCTATTTTCACAAAGTTCTCCAAAAAATGTGAT
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                                                  1150
GATCAAAACTAGGAATTAGTGTTCTGTGTCTTAGGCCCTAAAATCTTCCT
                                                  1200
GTGAATTCCATTTTTAAGGTAGTCGAGGTGAACCGCGTCTGGTCTGCAGA
                                                  1250
GGATAGAAAAAAGGCCCTCTGATACCTCAAGTTAGTTTCACCTTTAAAGA
                                                  1300
AGGTCGGAAGTAAAGACGCAAAGCCTTTCCCGGACGTGCGGAAGGGCAAC
                                                  1350
GTCCTTCCTCATGGCCGGAAATGGAACTTTAATTTCCCGTTCCCCCCAAC
                                                  1400
CAGCCCGCCCGAGAGAGTGACTCTCACGAGAGCCGCGAGAGTCAGCTTGG
                                                  1450
CCAATCCGTGCGGTCGGCGGCCGCTCCCTTTATAAGCCGACTCGCCCGGC
                                                  1500
AGCGCACCGGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGCCATTTTT
                                                  1550
TGTCTAACCCTAACTGAGAAGGGCGTAGGCGCCGTGCTTTTGCTCCCCGC
                                                  1600
GCGCTGTTTTTCTCGCTGACTTTCAGCGGGCGGAAAAGCCTCGGCCTGCC
                                                  1650
GCCTTCCACCGTTCATTCTAGAGCAAACAAAAAATGTCAGCTGCTGGCCC
                                                  1700
GTTCGCCCCTCCCGGGGACCTGCGGCGGGTCGCCTGCCCAGCCCCCGAAC
                                                  1750
CCCGCCTGGAGGCCGCGGTCGGCCCGGGGCTTCTCCGGAGGCACCCACTG
                                                  1800
CCACCGCGAAGAGTTGGGCTCTGTCAGCCGCGGGTCTCTCGGGGGCGAGG
                                                  1850
GCGAGGTTCAGGCCTTTCAGGCCGCAGGAAGAGGAACGGAGCGAGTCCCC
                                                  1900
GCGCGCGGCGCGATTCCCTGAGCTGTGGGACGTGCACCCAGGACTCGGCT
                                                  1950
CACACATGCAGTTCGCTTTCCTGTTGGTGGGGGGAACGCCGATCGTGCGC
                                                  2000
ATCCGTCACCCCTCGCCGGCAGTGGGGGCTTGTGAACCCCCAAACCTGAC
                                                  2050
TGACTGGGCCAGTGTGCTGCAAATTGGCAGGAGACGTGAAGGCACCTCCA
                                                  2100
AAGTCGGCCAAAATGAATGGGCAGTGAGCCGGGGTTGCCTGGAGCCGTTC
                                                  2150
CTGCGTGGGTTCTCCCGTCTTCCGCTTTTTGTTGCCTTTTATGGTTGTAT
                                                  2200
TACAACTTAGTTCCTGCTCTGCAGATTTTGTTGAGGTTTTTGCTTCTCCC
                                                  2250
AAGGTAGATCTCGACCAGTCCCTCAACGGGGTGTGGGAGAACAGTCATT
                                                  2300
TTTTTTTGAGAGATCATTTAACATTTAATGAATATTTAATTAGAAGATCT
                                                  2350
AAATGAACATTGGAAATTGTGTTCCTTTAATGGTCATCGGTTTATGCCAG
                                                  2400
AGGTTAGAAGTTTCTTTTTTGAAAAATTAGACCTTGGCGATGACCTTGAG
                          2426
CAGTAGGATATAACCCCCACAAGCTT     (SEQ ID NO:1)
```

The RNA component sequence begins at base 1459. A variety of transcriptional control elements can also be identified in the sequence. An A/T (TATA) box consensus sequence is found at nucleotides 1438-1444, PSE consensus sequences are found at nucleotides 1238-1259 as well as nucleotides 1406-1414, a CAAT box consensus sequence is found at nucleotides 1399-1406; an SP1 consensus sequence is found at nucleotides 1354-1359 and a β-interferon response element consensus sequence is found at nucleotides 1234-1245.

Plasmid pGRN33 contains an ~2.5 kb HindIII-SacI insert containing sequences from lambda clone 28-1 that contain the sequence of hTR. Plasmid pGRN33 was deposited with the American Type Culture Collection pursuant to the Budapest Treaty and granted accession number ATCC 75926. A PstI fragment of the ~2.4 kb SauIIIA1-HindIII fragment of clone 28-1 also contains the hTR sequence. The sequence of the PstI fragment, determined upon re-sequencing, is provided in SEQ ID NO:2, below:

```
   1 CTGCAGAGGATAGAAAAAAGGCCCTCTGATACCTCAAGTTAGTTTCACCTTTAAAGAAGG
     GACGTCTCCTATCTTTTTTCCGGGAGACTATGGAGTTCAATCAAAGTGGAAATTTCTTCC
     -PST1-

  61 TCGGAAGTAAAGACGCAAAGCCTTTCCCGGACGTGCGGAAGGGCAACGTCCTTCCTCATG
     AGCCTTCATTTCTGCGTTTCGGAAAGGGCCTGCACGCCTTCCCGTTGCAGGAAGGAGTAC

 121 GCCGGAAATGGAACTTTAATTTCCCGTTCCCCCCAACCAGCCCGCCCGAGAGAGTGACTC
     CGGCCTTTACCTTGAAATTAAAGGGCAAGGGGGGTTGGTCGGGCGGGCTCTCTCACTGAG

 181 TCACGAGAGCCGCGAGAGTCAGCTTGGCCAATCCGTGCGGTCGGCGGCCGCTCCCTTTAT
     AGTGCTCTCGGCGCTCTCAGTCGAACCGGTTAGGCACGCCAGCCGCCGGCGAGGGAAATA

                                5' HTR
                                **********************************
 241 AAGCCGACTCGCCCGGCAGCGCACCGGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGC
     TTCGGCTGAGCGGGCCGTCGCGTGGCCCAACGCCTCCCACCCGGACCCTCCCCACCACCG

                TEMPLATE
     **********************************************************
 301 CATTTTTTGTCTAACCCTAACTGAGAAGGGCGTAGGCGCCGTGCTTTTGCTCCCCGCGCG
     GTAAAAAACAGATTGGGATTGACTCTTCCCGCATCCGCGGCACGAAAACGAGGGGCGCGC

     **********************************************************
 361 CTGTTTTTCTCGCTGACTTTCAGCGGGCGGAAAAGCCTCGGCCTGCCGCCTTCCACCGTT
     GACAAAAAAGAGCGACTGAAAGTCGCCCGCCTTTTCGGAGCCGGACGGCGGAAGGTGGCAA

     **********************************************************
 421 CATTCTAGAGCAAACAAAAAATGTCAGCTGCTGGCCCGTTCGCCCCTCCCGGGGACCTGC
     GTAAGATCTCGTTTGTTTTTTTACAGTCGACGACCGGGCAAGCGGGGAGGGCCCCTGGACG

     **********************************************************
 481 GGCGGGTCGCCTGCCCAGCCCCCGAACCCCGCCTGGAGGCCGCGGTCGGCCCGGGGCTTC
     CCGCCCAGCGGACGGGTCGGGGGCTTGGGGCGGACCTCCGGCGCCAGCCGGGCCCCGAAG
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          **********************************************************
541 TCCGGAGGCACCCACTGCCACCGCGAAGAGTTGGGCTCTGTCAGCCGCGGGTCTCTCGGG
    AGGCCTCCGTGGGTGACGGTGGCGCTTCTCAACCCGAGACAGTCGGCGCCCAGAGAGCCC

          **********************************************************
601 GGCGAGGGCGAGGTTCAGGCCTTTCAGGCCGCAGGAAGAGGAACGGAGCGAGTCCCCGCG
    CCGCTCCCGCTCCAAGTCCGGAAAGTCCGGCGTCCTTCTCCTTGCCTCGCTCAGGGGCGC

          **********************************************************>
661 CGCGGCGCGATTCCCTGAGCTGTGGGACGTGCACCCAGGACTCGGCTCACACATGCAGTT
    GCGCCGCGCTAAGGGACTCGACACCCTGCACGTGGGTCCTGAGCCGAGTGTGTACGTCAA

721 CGCTTTCCTGTTGGTGGGGGGAACGCCGATCGTGCGCATCCGTCACCCCTCGCCGGCAGT
    GCGAAAGGACAACCACCCCCCTTGCGGCTAGCACGCGTAGGCAGTGGGGAGCGGCCGTCA

781 GGGGGCTTGTGAACCCCCAAACCTGACTGACTGGGCCAGTGTGCTGCAAATTGGCAGGAG
    CCCCCGAACACTTGGGGGTTTGGACTGACTGACCCGGTCACACGACGTTTAACCGTCCTC

841 ACGTGAAGGCACCTCCAAAGTCGGCCAAAATGAATGGGCAGTGAGCCGGGGTTGCCTGGA
    TGCACTTCCGTGGAGGTTTCAGCCGGTTTTACTTACCCGTCACTCGGCCCCAACGGACCT

901 GCCGTTCCTGCGTGGGTTCTCCCGTCTTCCGCTTTTTGTTGCCTTTTATGGTTGTATTAC
    CGGCAAGGACGCACCCAAGAGGGCAGAAGGCGAAAAACAACGGAAAATACCAACATAATG

961 AACTTAGTTCCTGCTCTGCAG
    TTGAATCAAGGACGAGACGTC
                  ^
                 976
                 PST1
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D. *Reporter Polynucleotide*

Telomerase reporter constructs also include a nucleotide sequence encoding a reporter polynucleotide operably linked to the transcription regulatory region. The reporter polynucleotide is heterologous to the transcription regulatory region. That is, the reporter polynucleotide is not naturally under the control of the transcription regulatory region. The reporter polynucleotide is selected so that its expression is detectable. Detection can be direct, such as detection of the polynucleotide, itself, through, e.g., hybridization, or detection of a protein encoded by the polynucleotide by, e.g., immunoassay or visible detection (e.g., fluorescence), or indirectly by the expression of a phenotype (e.g., drug resistance.)

The reporter polynucleotide sequence can comprise a selectable marker gene (e.g., neo, HPRT, screenable cell surface protein, auxotrophic gene), a detectable gene product (e.g., luciferase, fluorescent protein, β-galactosidase, a hybridizable transcript having a predetermined sequence complementary to a probe or primer), or the like. A reporter polynucleotide is suitable so long as its transcription provides a basis for reporting transcription,

and, for certain embodiments, preferably also for identifying and selecting cells in which the reporter polynucleotide sequence is substantially transcribed.

A reporter gene or other structural gene is preferably inserted in operable linkage with the hTR gene upstream enhancer (and optionally including the hTR promoter). The reporter gene (or other structural gene) is positioned to ensure correct transcription and translation according to standard cloning methods in the art. A targeting construct may be produced having recombinogenic homology regions flanking the reporter gene (or other structural gene) which correspond to the sequences flanking the chosen insertion site, which will be downstream of the transcription start site. A transgene comprising the regulatory sequences identified herein as the hTR transcription regulatory region can also be produced, however it may be desirable to include additional sequences upstream or downstream of the hTR transcription start site; such sequences can be readily isolated by routine "chromosome walking" screening of a human genomic library.

The polynucleotide sequence encoding a reporter polynucleotide is operably linked to cis-acting transcriptional regulatory sequences (e.g., promoter, enhancer) of a telomerase gene (e.g., hTR), so that the reporter polynucleotide is expressed in telomerase-expressing cells in a manner similar to the expression of the endogenous hTR gene in naturally-occurring cells of the same or equivalent type, preferably neoplastic or immortal cells, stem cells, or germ cells. Thus, it is usually preferable to operably link a reporter sequence to transcriptional regulatory elements which naturally occur in or near the telomerase gene (e.g., hTR gene).

E. *Operable Linkage*

Transgenes and expression polynucleotides of the invention comprise a transcriptional regulatory sequence of a telomerase gene operably linked to a reporter gene or other structural gene. Targeting constructs of the invention may comprise such a transcriptional regulatory sequence. Suitable transcriptional regulatory sequences are those which confer telomerase-specific transcription of the linked gene, although low levels of transcription may occur

in a cell which do not express telomerase so long as such leaky or background expression does not substantially interfere.

The operable linkage between transcription regulatory region and nucleotide sequence encoding the reporter polynucleotide may be formed by homologous sequence targeting to place the reporter gene downstream of (i.e., towards the carboxy-terminus of the encoded naturally-occurring polypeptide in translational reading frame orientation) a transcriptional regulatory sequence (i.e., a promoter and the additional elements which confer specific cell-type expression) of the endogenous telomerase gene.

Alternatively, the operable linkage may be formed exogenously as a transgene, wherein the reporter gene is operably linked to a transcriptional regulatory sequence isolated from an endogenous telomerase gene, typically by genomic DNA cloning.

IV. *RECOMBINANT HOST CELLS*

A host cell comprising a telomerase reporter construct can be made from a variety of suitable cell types. The telomerase reporter construct can be present as a polynucleotide sequence in the genome of the cell (e.g., homologously integrated into a predetermined chromosomal locus or non-homologously integrated), can be present as a replicable episome (e.g., as a plasmid, viral genome, or artificial chromosome), or can be present as a non-replicable episome, such as for transient expression assays. In an aspect, the host cell is a eukaryotic cell, for example a yeast cell, an insect cell, or a mammalian cell. In an aspect, the host cell is a mammalian cell that expresses the endogenous, naturally-occurring telomerase gene, and may express detectable telomerase activity. In an aspect, the host cell is a mammalian cell that substantially does not transcribe the endogenous, naturally-occurring telomerase gene, and substantially lacks detectable telomerase activity. In an aspect, the host cell is a mammalian cell that detectably transcribes the endogenous, naturally-occurring telomerase gene, and has detectable telomerase activity. In a preferred aspect, the host cell is a human cell.

V. *TRANSGENIC ANIMALS*

Transgenic non-human animal comprising a genomic copy of a telomerase reporter construct can be made by those skilled in the art. In a variation, the telomerase reporter construct is present in the genome of the animal as a germline copy, or multiple copies, which are non-homologously integrated into one or more chromosomal locus. In a variation, the telomerase reporter construct is present in the genome of the animal as a germline copy, or multiple copies, which are homologously integrated into a chromosomal locus. In a variation, the telomerase reporter construct is present in the genome of a subset of somatic cells the animal and is substantially not present in germ cells. In a variation, the telomerase reporter construct is present in the genome of cells of one organ, tissue, or cell type of the animal and is substantially absent in other cells of the animal.

Genomic clones of hTR, particularly of the murine or human hTR gene, may be used to construct homologous targeting constructs for generating cells and transgenic non-human animals having at least one functionally disrupted hTR allele. Guidance for construction of homologous targeting constructs may be found in the art, including: Rahemtulla et al. (1991) *Nature* 353: 180; Jasin et al. (1990) *Genes* Devel. 4: 157; Koh et al. (1992) *Science* 256: 1210; Molina et al. (1992) *Nature* 357: 161; Grusby et al. (1991) *Science* 253: 1417; Bradley et al. (1992) *Bio/Technology* 10: 534. Homologous targeting can be used to generate so-called "knockout" mice, which are heterozygous or homozygous for an inactivated hTR allele. Such mice may be sold commercially as research animals for investigation of immune system development, neoplasia, spermatogenesis, may be used as pets, may be used for animal protein (foodstuff), and other uses.

VI. *METHODS FOR IDENTIFYING TELOMERASE TRANSCRIPTION* MODULATORS

A method for identifying an agent which modulates transcription of a telomerase reporter construct typically comprises determining the ability of an agent, typically a predetermined agent, to modulate transcription of the reporter polynucleotide sequence of said telomerase reporter construct in a transcription

assay, which is generally: (1) an *in vitro* transcription reaction having a telomerase reporter construct, (2) a stably or transiently transfected host cell having a telomerase reporter construct, or (3) a transgenic animal having a genome having a telomerase reporter construct; wherein modulation is defined as a reproducible and detectable increase or decrease in expression of the reporter polynucleotide sequence in the presence of said agent as compared to a comparable transcription assay lacking said agent. Expression can be detected directly or indirectly, as discussed, e.g., by actual detection of the reporter polynucleotide or measurement of a phenotypic trait conferred by the reporter polynucleotide. Incubation conditions for obtaining transcription are generally physiological conditions. Such conditions are well known in the art for both *in vitro* and *in vivo* assays.

In an embodiment, an agent is introduced into or administered to a transcription assay wherein sequence-specific transcriptional regulation of a reporter gene is effected by a predetermined cis-acting operably linked mammalian telomerase transcription regulatory region.

Agents for testing in the assay may be randomly screened using any of a variety of combinatorial libraries or agents pre-selected for their ability to modulate a signal in a functional pathway that regulates expression of a mammalian telomerase gene. This includes, for example, hormonal signals and expression of telomerase-specific transcription factors.

In certain embodiments, the transcription regulatory region is telomerase-expressing cell specific to the cell in which the assay is being carried out. Such regions are particularly useful for identifying drug candidates for specific inhibition of the telomerase gene, without harmful inhibition of other cell functions.

Agents identified by these assays that alter transcription from a transcription regulatory region of a mammalian telomerase gene are candidates for further drug testing for safety and efficacy.

VII. *THERAPEUTIC AND PROPHYLACTIC ASPECTS*

Modulating expression of telomerase is useful in the prophylactic or therapeutic treatment of telomerase-related conditions. For example, inhibiting

telomerase activity in cancer cells inhibits their continued growth. Inhibiting telomerase activity in germ-line cells is useful in inhibiting fertility. Activating telomerase activity in hematopoietic cells promotes continued production of blood cells. Accordingly, agents identified by the assays of this invention are useful in modulating telomerase activity in therapeutic and prophylactic treatments, e.g., as anti-neoplastic agents.

Because telomerase is primarily active in tumor, germline, and certain stem cells, for example stem cells of the hematopoietic system, other normal cells are not affected by telomerase inhibition therapy using telomerase transcription modulators of the present invention. Steps can also be taken to avoid contact of the telomerase inhibitor with germline or stem cells, although this may not be essential. For instance, because germline cells express telomerase activity, inhibition of telomerase may negatively impact spermatogenesis and sperm viability, and telomerase inhibitors may be effective contraceptives or sterilization agents. This contraceptive effect may not be desired, however, by a patient receiving a telomerase inhibitor of the invention for treatment of cancer. In such cases, one can deliver a telomerase inhibitor of the invention in a manner that ensures the inhibitor will only be produced during the period of therapy, such that the negative impact on germline cells is only transient, or more localized administration can be used.

These methods can be carried out by delivering to a patient, more particularly to diseased cells, a functional telomerase transcriptional modulator of the invention to the cell. For instance, the agent can be delivered in a liposome or other delivery enhancement formulation.

Telomerase transcriptional modulators can be used to stimulate telomerase activity in various human cells that otherwise lack detectable telomerase activity due to low levels of expression of the RNA component or a protein component of telomerase. If the telomerase RNA component is not sufficient to stimulate telomerase activity, then the agent can be introduced along with genes expressing the protein components of telomerase to stimulate telomerase activity. Thus, the invention provides methods for treating a condition associated with the telomerase activity within a cell or group of cells

by contacting the cell(s) with a therapeutically effective amount of a telomerase transcription modulator that alters telomerase activity in that cell.

Cells that incorporate agents having the property of stimulating hTR gene expression from the promoter of the telomerase RNA gene can exhibit an increase in telomerase activity and an associated extended replicative life span. Such therapy can be carried out *ex vivo* on cells for subsequent introduction into a host or can be carried out *in vivo*. The advantages of stabilizing or increasing telomere length by adding the telomerase transcription modulator *ex vivo* to normal diploid cells include: telomere stabilization can arrest cellular senescence and allow potentially unlimited proliferative capacity of the cells; and normal diploid cells with an extended life span can be cultured *in* vitro for drug testing, virus manufacture, transplantation, or other useful purposes. In particular, *ex vivo* amplified stem cells of various types can be used in cell therapy for particular diseases, as noted above. Telomere stabilization can also suppress cancer incidence in replicating cells by preventing telomeres from becoming critically short as cells near crisis.

Cells that can be treated with telomerase gene transcription modulators that activate hTR transcription include but are not limited to hematopoietic stem cells (viral infections, including HIV infection leading to AIDS and post-chemotherapy), vascular endothelial cells (including cardiac and cerebral forms), skin fibroblasts and basal skin keratinocytes (wound healing and burns), chondrocytes (arthritis), brain astrocytes and microglial cells (Alzheimer's Disease), osteoblasts (osteoporosis), retinal cells (eye diseases), and pancreatic islet cells (Type I diabetes).

Typically, the therapeutic methods of the invention involve the administration of a telomerase transcription modulator that functions to inhibit or stimulate telomerase activity under *in vivo* physiological conditions and is sufficiently stable under those conditions.

In related aspects, the invention features pharmaceutical compositions that include a pharmacologically effective amount of a telomerase transcriptional modulator of the invention, e.g., an amount effective to inhibit or stimulate telomerase expression sufficient to result in the prophylactic or therapeutic treatment. Pharmaceutical compositions of telomerase transcriptional

modulators, or combinations of such species and/or with other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a telomerase transcription activator preparation.

The therapeutic agent can be provided in a formulation suitable for parenteral, nasal, oral, or other mode of administration.

In another aspect of the invention, buffered aqueous solutions comprising at least one telomerase-inhibitory or activating species of the invention at a concentration of at least 1 nM but not more than about 100 mM is formulated for administration, usually at a concentration of about 0.1 to 10 mM, typically by intravenous route, to a patient undergoing anti-neoplastic or anti-helminthic chemotherapy. The buffered aqueous solutions of the invention may also be used, typically in conjunction with other established methods, for organ culture, cell culture, delivery to transformed cells, and *ex vivo* cellular therapies. Nonaqueous formulations, such as lipid-based formulations are also provided, including stabilized emulsions. The telomerase-modulating compositions are administered by various routes, including intravenous injection, intramuscular injection, subdermal injection, intrapericardial injection, surgical irrigation, topical application, ophthalmologic application, lavage, gavage, enema, intraperitoneal infusion, mist inhalation, oral rinse, and other routes, depending upon the specific medical or veterinary use intended.

The telomerase-modulating species of the present invention can be administered as a pharmaceutical composition comprising the compound in combination with a pharmaceutically acceptable excipient. Such excipients include water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol, phosphate, acetate, gelatin, collagen, and the like. One may additionally include other suitable preservatives, stabilizers and antimicrobials, antioxidants, buffering agents and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences*, 19th Ed., (1995) Mack Publishing Co. Typically, a modulator of the invention is formed in a pharmaceutical dosage form comprising an excipient and not less than 1 μg nor more than about 100 grams of at least one telomerase-modulating species of the invention. In another aspect of the invention, buffered aqueous

solutions comprising at least one telomerase-modulating species of the invention at a concentration of at least 1 nM but generally not more than about 100 mM is formulated for administration, usually at a concentration of about 0.1 μM to 10 mM, typically by intravenous route or via in infusion pump for localized delivery (e.g., to a solid tumor) or sustained dosing.

Alternatively, one may incorporate or encapsulate the telomerase-modulating agents in a suitable polymer matrix, liposome or membrane, thus providing a sustained release delivery device suitable for implantation near the site to be treated locally. In general, with sustained release delivery, the formulations are constructed so as to achieve a constant concentration which will be bioequivalent to about 100 times the serum level or 10 times the tissue concentration. Nonaqueous formulations, such as lipid-based formulations are also provided, including stabilized emulsions.

The amount of telomerase-modulating agent required to treat any particular neural disorder will of course vary depending on the nature and severity of the disorder, the age and condition of the patient, and other factors readily determined by one of skill in the art. Suitable dosages are from 1 ng/kg to about 1000 mg/kg, more preferably 1 μg/kg to about 100 mg/kg.

VIII. *TRANSGENES*

Additionally, a hTR cDNA or genomic gene copy may be used to construct transgenes for expressing hTR at high levels and/or under the transcriptional control of transcription control sequences which do not naturally occur adjacent to the hTR gene. For example but not limitation, a constitutive promoter (e.g., a HSV-*tk* or *pgk* promoter) or a cell-lineage specific transcriptional regulatory sequence (e.g., a CD4 or CD8 gene promoter/enhancer) may be operably linked to a hTR polynucleotide sequence to form a transgene (typically in combination with a selectable marker such as a *neo* gene expression cassette). Such transgenes can be introduced into cells (e.g., ES cells, hematopoietic stem cells) and transgenic cells and transgenic nonhuman animals may be obtained according to conventional methods.

Alternatively, an hTR transcription regulatory region can be linked to a structural gene or a reporter polynucleotide to form a transgene which can be transferred into a cell, cell line, or non-human animal germline.

IX. *TELOMERASE-DEPENDENT GENES*

Expression of recombinant hTR in cells, particularly cells which are naturally telomerase negative, may be used to identify and isolate genes that are transcriptionally modulated, either positively or negatively, by the presence of hTR. Such genes are typically initially identified as cDNA clones isolated from subtractive cDNA libraries, wherein RNA isolated from cells expressing recombinant hTR and RNA isolated from control cells (i.e., not expressing recombinant hTR) are used to generate the subtractive libraries and screening probes. In such a manner, hTR-dependent genes may be isolated. hTR-dependent genes (or their regulatory sequences operably linked to a reporter gene) may be used as a component of an *in vitro* transcription assay; such transcription assays may be used to screen for agents which inhibit hTR-dependent gene transcription and are thereby identified as candidate telomerase modulatory agents.

In vitro transcription reactions are typically performed by conventional methods, wherein the transcription template is a reporter polynucleotide operably linked and under transcriptional control of a telomerase gene transcription regulatory region (e.g., the hTR gene promoter region). A modification of standard *in vitro* transcription cocktails is the use of a cell extract or nuclear extract from cells which express the telomerase gene (e.g., hTR gene). In an embodiment, the *in vitro* cocktail comprises labeled ribonucleotide triphosphates, a telomerase gene transcription template, a nuclear extract from HT1080 cells, and physiological conditions.

X. *PURIFICATION OF MAMMALIAN TELOMERASE PROTEIN COMPONENT*

Mammalian telomerase protein component can be purified from telomerase-expressing cells, such as HT1080 cells, 293 cells, and other suitable immortalized cell lines by the method disclosed in Weinrich et al., U.S. Patent

Application 08/288,501, filed 10 August 1994 and Weinrich et al., International application PCT/US97/06012, filed April 4, 1997. For example and not limitation, human telomerase can be purified from cell extracts. Mammalian telomerase extracts can be stripped of the telomerase RNA component, if desired, by treatment with an RNAse activity or other suitable means to dissociate and/or degrade the telomerase RNA component while leaving telomerase protein component substantially intact and capable of reconstitution with addition of exogenous telomerase RNA component, or a mimetic thereof such as may be produced recombinantly or the like. The telomerase protein component thus purified, and optionally stripped of endogenous telomerase RNA component, can be used in the agent screening assays described herein, and for other uses.

XI. *TAGGED TELOMERASE RNA COMPONENT CONSTRUCTS*

This invention provides tagged RNA component constructs for determining whether an agent inhibits the association of a mammalian RNA component of telomerase with a protein component of telomerase. Constructs of this aspect of the invention comprise an expression control sequence (e.g., transcription regulatory region) operably linked to a nucleotide sequence coding for the expression of a fusion polynucleotide comprising a mammalian RNA component of telomerase and a tag sequence.

The expression control sequence preferably is heterologous to the sequence encoding a mammalian RNA component of telomerase. For example, the region can comprise a constitutive promoter or inducible promoter and is, preferably a high expression promoter.

The mammalian RNA component of telomerase can be hTR or the RNA component of any other mammal, such as a mouse, etc.

A "tag sequence" is a sequence that is capable of specific recognition by a binding substance. For example, the tag sequence can be a sequence that is specifically recognized by an antibody. Also, the tag sequence can code for the expression of the sequence of a DNA binding domain, such as the *tar* sequence which is recognizable by *tat*. The ability of the tag sequence to be recognized allows one to isolate the entire transcript and any molecules bound

to it, specifically, protein components of telomerase. The sequences encoding the tag sequence can be placed either up-stream or down-stream of the sequence coding for the expression of the RNA component of telomerase. Upon expression the construct produces a tagged telomerase RNA component.

The construct, telomerase protein component and the agent typically are contacted under physiological conditions. In one embodiment, the physiological conditions are *in vitro* conditions. In another embodiment, the physiological conditions are conditions inside a cultured cell. That is, a cell that expresses the protein component of telomerase (either naturally or recombinantly) is transfected with an expression cassette that expressed the tagged construct. The agent is contacted with the cell or, e.g., a cell extract. Binding between the tagged construct and the telomerase protein component is detected. A side-by-side control to which no agent has been added can be used as a comparison to measure modulation in the amount of binding between the telomerase protein component and the tagged telomerase RNA component construct.

XII. *METHODS OF DETERMINING WHETHER AN AGENT INHIBITS ASSOCIATION BETWEEN A MAMMALIAN TELOMERASE RNA COMPONENT AND A PROTEIN COMPONENT OF TELOMERASE*

This invention provides methods of determining whether an agent inhibits association between a mammalian RNA component of telomerase and a protein component of telomerase. The methods involve contacting a cell that expresses a tagged RNA component of telomerase and a protein component of telomerase with an agent, capturing the tagged RNA component of telomerase and determining whether the amount of protein bound to the tagged RNA component is different than the amount bound in the absence of the agent. A decreased amount of bound protein indicates that the agent inhibits the association of the RNA component of telomerase with a protein component of telomerase. An increased amount of bound protein indicates that the agent promotes the association of the RNA component with a protein component of telomerase. One means of determining the amount of bound protein is by using immunoassays involving antibodies that recognize protein components of telomerase.

XIII. *POSITION-EFFECT MODULATORY AGENTS*

Telomeric repeats organize a change in chromatin structure that propagates thousands of base pairs into the subtelomeric region. This altered telomeric chromatin (termed chromatel) is believed to be the actual regulator of genes that control life span. In the Chromatel Model of Cellular Senescence, chromatel extension is closely linked to the number of telomeric repeats, so as telomeres shorten with replicative senescence, chromatels also shorten. As with other chromatin position effects, many other proteins and factors besides telomere length affect the extension of chromatels. According to the Chromatel Model, therefore, heterochromatin proteins as well as histone and DNA modification affect chromatel extension and, by implication, cell life span.

As an example of histone modifications, histone hyper-acetylation is known to weaken heterochromatin position effects and should cause a shortening in the chromatel for the same size telomeres. Histone hyper-acetylation is believed to accelerate senescence. Inhibitors of histone poly-ADP-ribosylation, which stabilize chromatel extension, lead to extended cell life span.

Evidence that telomere length is linked to changes in chromatin acetylation can be shown by inducing hyper-acetylation in telomerase-positive cell lines (HT1080 and 293) and then checking telomere length after some 10-30 doublings. The cell will attempt to maintain chromatel length by activating telomerase and lengthening telomeres to counteract the shortening of chromatels by hyper-acetylation. Telomere lengthening in cells with hyper-acetylated histones indicates therapeutic approaches (e.g. cells can be treated with an inhibitor of histone deacetylase and extend their telomeres to extend life-span, such as in transgenic mice).

In view of this, a novel therapeutic strategy for telomerase inhibition in cancer is possible: telomerase inhibition combined with a suppressor of chromatin position effects kills cancer cells much faster than telomerase inhibition alone. Cancer cells are still preferentially affected by this two-drug treatment, because histone acetylation only induces premature senescence in dividing cells (chromatels shortened by histone acetylation are presumably fixed by changes in DNA methylation during division).

Therefore, it is desirable to screen for genes or small molecules that affect chromatin position effects and then test their effect on cell life span.

Certain agents are known to inhibit position effect, resulting in increased expression of genes whose expression is dimished by the position effect. In general, these are agents that either stabilize euchromatin or destabilize heterochromatin. Such agents include agents that hyper-acetylate histones, agents that inhibit deacetylaces, agents that inhibit histone poly-ADP-ribosylation, agents that bind to histones and intercalating agents.

Cells wherein chromatin position conditionally represses expression of a reporter polynucleotide that is integrated or homologously recombined into a chromosomal locus can be made. A reporter polynucleotide comprises a polynucleotide sequence encoding a selectable drug marker gene (e.g., neo, HPRT, etc.) under transcriptional control of an operably linked transcription regulatory region which comprises an hTR promoter or which typically is not derived from a mammalian telomerase gene and often is substantially constitutively active in a mammalian cell line (e.g., an SV40 large T Ag promoter/enhancer, GAPDH promoter, etc.). The construct can comprise more than one selectable drug marker -- one whose expression can be positively selected and one whose expression can be negatively selected. The construct also can further comprise a sequence encoding a reporter polynucleotide that is not a selectable drug marker. The reporter polynucleotide is introduced into a cell population, such as a cultured cell line or organism, and the cell population is cultured in the presence of an agent predetermined to reduce position effect (e.g., 0.5 mM sodium butyrate) and the cell population is exposed to a positive selection agent (e.g, G-418 for neo^R; HAT medium for positive selection of HPRT-expressing cells; gancyclovir for tk-expressing cells, and the like) and the population is selected for cells expressing the presence of the drug marker. The selected population is cultured in the substantial absence of said agent predetermined to reduce position effect, and the cell population is exposed to a negative selection agent (e.g., FIAU for tk-expressing cells, 6-thioguanine for HPRT-expressing cells) which selects for cells which substantially lack expression of the drug marker.

The resultant cell doubly-selected subpopulation is enriched in cells which have integrated reporter polynucleotides exhibiting position effect, wherein the reporter polynucleotide is expressed under conditions where position effect is relieved (e.g., 0.5 mM sodium butyrate), but is substantially transcriptionally repressed by position effect in the absence of agents which relieve position effect. The doubly-selected cell population typically comprises a collection of cell clones which have integrated reporter polynucleotides into position effect-sensitive chromatin regions; these cells are termed position effect reporter cells. In a variation, individual clonal progeny of position effect reporter cells are selected from the doubly-selected cell population for subsequent use.

Agents which modulate chromatin position effects on expression of a reporter polynucleotide that is integrated or homologously recombined into a chromosomal locus can be identified. Position effect reporter cells are cultured in conditions wherein the integrated reporter polynucleotide is substantially transcriptionally silent, the cells are exposed to an agent, and transcription of the reporter polynucleotide is determined (e.g., by positive selection, negative selection, RNA transcript analysis, or other phenotypic determination or assay). An agent which produces a detectable increase in transcription of the reporter polynucleotide as compared to the position effect reporter cells cultured in the absence of the agent is thereby identified as a position effect modulatory agent, and more specifically as position effect antagonists.

In a variation, position effect cells are cultured under conditions where position effect is relieved (e.g., 0.5 mM sodium butyrate), the cells are exposed to an agent, and transcription of the reporter polynucleotide is determined (e.g., by positive selection, negative selection, RNA transcript analysis, or other phenotypic determination or assay). An agent which produces a detectable decrease in transcription of the reporter polynucleotide as compared to the position effect reporter cells cultured in the absence of the agent is thereby identified as a position effect modulatory agent, and more specifically as position effect agonists.

A cDNA expression library is introduced into a population of position effect reporter cells, whereby a library of position effect reporter cells is created wherein the position effect reporter cells individually express cDNA

library members, which can be in either sense or antisense orientation, or a combination of sense and antisense orientations. In this embodiment, the introduced cDNA expression library member can serve as the agent and expressed cDNA library members which produce a detectable decrease or increase in reporter polynucleotide transcription as compared to cells lacking a cDNA library member are thereby identified as position effect antagonists or agonists, respectively. In a variation, a predetermined position effect modulator is added to a telomerase assay and the effect on telomerase activity is determined. In a variation, a predetermined position effect modulator is administered to a cell which does not express telomerase in the absence of the agent and the ability of the agent to induce detectable telomerase is determined. In a variation, a predetermined position effect modulator is administered to a telomerase-expressing cell and the ability of the agent to repress telomerase activity is determined.

In an embodiment, the agent is a polynucleotide, such as an antisense RNA transcribed from an antisense cDNA expression library member transfected into a position effect reporter cell.

For chromatin position-effect screening, the SV40 or hTR promoter can be operably linked to the coding region of the HPRT marker gene, whose expression can be selected for in HAT media or selected against in 6-thioguanine. Both the SV40 and hTR promoters appear sensitive to chromatin position effects, so the choice between these two promoters should be empirical, depending on which gives best results. Stable transformants will be prepared using the HPRT expression vectors transfected into $HPRT^-$ cells and selected in 0.5 mM sodium butyrate to induce histone hyper-acetylation that weakens position effects and HAT media to select for HPRT expression. Individual HPRT-expressing colonies will then be shifted to 6-thioguanine media without butyrate to select for low HPRT expressing cells in conditions where position effects are strengthened in the absence of histone hyper-acetylation. Clonal populations that can switch HPRT expression on or off in the presence or absence of butyrate will be selected as potential indicator cells for position-effect screening. If histone hyper-acetylation appears linked to telomere length, then the indicator cells can be tested for linkage to telomeres by incubating the cells

with telomerase inhibitors that induce telomere loss. If telomerase inhibition causes a delayed reactivation of HPRT in some of the clones cultured without butyrate, then these clones would be the best indicator cells for chromatel position-effect screening (termed PES cells).

HPRT-switchable PES cells can be used in both gene and small-molecule screens for enhancers and suppressors of position effects. For example, to use the PES cells to screen for genes that suppress position effects, one would generate stable transformants of the PES cells by transfecting in a neo expression library containing antisense human gene tags (subtracted libraries with low levels of common sequences might be best) and by selection in HAT + G418 media in the absence of butyrate. DNA can be isolated from the colonies that grow out and the associated antisense human gene tags rescued by PCR amplification. The antisense tags that are pulled out in this screen can be added back to the PES cells to determine that the selected antisense tag was a real suppressor of position effects.

A special class of antisense genes that suppress position effects can be the telomerase proteins. Since antisense telomerase genes would only suppress position effects later as telomeres significantly shorten, a screen could be designed to catch this potential subclass of the position effect genes and rescue the clone before the cells die off from critically shortened telomeres. Like HPRT, URA3 is a gene marker that can be selected for and against to allow position effect cloning when the URA3 gene was placed in an appropriate position.

To select for genetic enhancers of chromatin position effects, one can generate stable transformants of the PES cells by transfecting in a neo expression library containing antisense human gene tags as before and then select in 6-thioguanine + G418 media in the presence of butyrate. This will select for antisense gene tags that enhance position effects or genes that in the sense orientation, would suppress position effects.

To screen for small molecules that enhance chromatin position effects, the PES cells will be cultured in 6-thioguanine in the presence of butyrate and various drugs to be tested. To screen for small molecule

suppressors of chromatin position effects, the PES cells will be cultured in HAT media in the absence of butyrate and the various agents to be tested.

Any genes or small molecules that alter chromatin position effects can be tested for effects on cell life span or normal cells. Enhancers of position effects would be expected to lengthen life span while suppressors would be expected to shorten it. Suppressors of chromatin position effects could be tested along with inhibitors of telomerase to determine whether tumor cell killing can be accelerated by this two-drug strategy. These secondary screens should provide novel therapeutics for cancer and age-related disease therapeutics.

The invention provides methods for treating a condition associated with the telomerase activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters position effect of gene sequences in that cell. Such telomerase-modulating agents are often small molecules (e.g., less than about 3000 Daltons) and can be used to modify telomerase activity *in vitro* and *in vivo*, frequently for therapeutic effect, and are used as laboratory reagents and/or pharmaceuticals.

In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a pharmaceutically acceptable carrier or salt, which may include formulation in a lipofection complex, liposome, or immunoliposome for targeted delivery of the therapeutic agent. The invention also provides combinations of such telomerase-mediated therapeutic agents with other pharmaceuticals, such as antineoplastic agents and other cytotoxic or cytostatic agents; antifungal agents (e.g., for treatment of AIDS patients); nucleotides, nucleosides, and analogs thereof; and other pharmaceutical agents suitable for treating disease conditions such as neoplasia, hyperplasia, HIV-infection/AIDS and associated pathologies, and other diseases characterized by abnormal telomere metabolism.

The present invention provides novel inventions. While specific examples have been provided, the above description is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The scope of the invention should, therefore, be determined not with reference to the above description, but

instead should be determined with reference to the appended claims along with their full scope of equivalents.

All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

WHAT IS CLAIMED IS:

1. A telomerase reporter construct recombinant polynucleotide comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence encoding a reporter polynucleotide heterologous to the transcription regulatory region, wherein the transcription regulatory region comprises nucleotide sequences sufficient for activating transcription of the reporter polynucleotide and wherein expression of the reporter polynucleotide is detectable.

2. The telomerase reporter construct of claim 1 wherein the transcription regulatory region of a mammalian telomerase component gene is a transcription regulatory region of a mammalian telomerase RNA component gene.

3. The telomerase reporter construct of claim 2 wherein the transcription regulatory region of a mammalian telomerase RNA component gene is a transcription regulatory region of a human telomerase RNA component ("hTR") gene locus.

4. The telomerase reporter construct of claim 3 wherein the transcription regulatory region comprises at least a TATA box consensus sequence and a CCAAT box consensus sequence of the hTR gene locus.

5. The telomerase reporter construct of claim 4 wherein the transcription regulatory region is hTR-expressing cell specific.

6. The telomerase reporter construct of claim 5 wherein the transcription regulatory region comprises a contiguous sequence of at least 1.4 kb upstream of nucleotide 1459 of SEQ ID NO:1.

7. The telomerase reporter construct of claim 5 wherein the transcription regulatory region comprises a contiguous sequence of at least 5 kb upstream of nucleotide 1459 of SEQ ID NO:1.

8. The telomerase reporter construct of claim 5 wherein the transcription regulatory region comprises a contiguous sequence of at least 10 kb upstream of nucleotide 1459 of SEQ ID NO:1.

9. The telomerase reporter construct of claim 5 wherein the reporter polynucleotide sequence encodes a selectable drug marker, β-galactosidase, fluorescent protein, chloramphenicol acetyltransferase or a sequence that specifically hybridizes to a preselected oligonucleotide probe.

10. A recombinant host cell comprising a telomerase reporter construct recombinant polynucleotide comprising a telomerase reporter construct recombinant polynucleotide comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence encoding a reporter polynucleotide heterologous to the transcription regulatory region, wherein the transcription regulatory region comprises nucleotide sequences sufficient for activating transcription of the reporter polynucleotide and wherein expression of the reporter polynucleotide is detectable.

11. The recombinant host cell of claim 10 wherein the transcription regulatory region of a mammalian telomerase component gene is a transcription regulatory region of a mammalian telomerase RNA component gene.

12. The recombinant host cell of claim 11 wherein the transcription regulatory region of a mammalian telomerase RNA component gene is a transcription regulatory region of a human telomerase RNA component ("hTR") gene locus.

13. The recombinant host cell of claim 12 wherein the transcription regulatory region is hTR-expressing cell specific.

14. A method for determining whether an agent modulates transcription of a nucleotide sequence operably linked to a transcription regulatory region of a mammalian telomerase gene comprising the steps of:

(a) incubating, under physiological conditions suitable for transcription, a telomerase reporter construct recombinant polynucleotide comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence encoding a reporter polynucleotide heterologous to the transcription regulatory region, wherein the transcription regulatory region comprises nucleotide sequences sufficient for activating transcription of the reporter polynucleotide and wherein expression of the reporter polynucleotide is detectable;

(b) contacting the construct with the agent;

(c) measuring the amount of expression of the reporter polypeptide from the construct; and

(d) determining whether the measured amount is different than a control amount of expression from the construct which has not been contacted with the agent;

whereby a difference between the measured amount and the control amount indicates that the agent modulates transcription from the transcription regulatory region.

15. The method of claim 14 wherein the transcription regulatory region of a mammalian telomerase component gene is a transcription regulatory region of a mammalian telomerase RNA component gene.

16. The method of claim 15 wherein the transcription regulatory region of a mammalian telomerase RNA component gene is a transcription regulatory region of a human telomerase RNA component ("hTR") gene locus.

17. The method of claim 14 conducted *in vitro*.

18. The method of claim 14 conducted *in vivo* wherein the step of incubating comprises incubating a recombinant host cell transfected with the telomerase reporter construct and wherein the telomerase regulatory region is telomerase-expressing cell specific to the recombinant host cell.

19. The method of claim 14 conducted *in vivo* and comprising the use of a transgenic animal having a genome having the telomerase reporter construct.

20. An agent that modulates transcription from a transcription regulatory region of a mammalian telomerase gene determined by the method of claim 14.

21. A method for prophylactic or therapeutic treatment of a telomerase-related condition comprising the step of administering to a subject a pharmacologically effective amount of an agent of claim 20.

22. A method for inhibiting the growth of a cell that expresses telomerase comprising transfecting the cell with an expression cassette comprising a transcription regulatory region of a mammalian telomerase gene operably linked to a nucleotide sequence coding for the expression of a product that inhibits growth of the cell.

23. A tagged RNA component construct recombinant polynucleotide comprising a transcription regulatory region operably linked to a nucleotide sequence encoding a tagged telomerase RNA component, wherein the component comprises a mammalian telomerase RNA component sequence and a tag sequence.

24. A method for determining whether an agent modulates association between a mammalian RNA telomerase component and a mammalian telomerase protein component comprising the steps of:

(a) contacting a tagged telomerase RNA component comprising a mammalian telomerase RNA component sequence and a tag sequence with a mammalian telomerase protein component and with an agent;

(b) measuring the amount of mammalian telomerase protein component bound to the tagged telomerase RNA component, and

(c) determining whether the measured amount is different than a control amount of binding between the mammalian telomerase protein component and the tagged telomerase RNA component which has not been contacted with the agent;

whereby a difference between the measured amount and the control amount indicates that the agent modulates binding between the mammalian RNA telomerase component and the telomerase protein component.

25. A method for generating position effect reporter cells comprising the steps of:

(a) introducing into a population of mammalian cells a reporter expression cassette comprising a transcription regulatory region operably linked to a nucleotide sequence encoding at least one selectable drug marker gene, whereby the reporter expression cassette is integrated or homologously recombined into a chromosome in a plurality of the cells;

(b) culturing the population in the presence of an agent that inhibits position effect;

(c) exposing the population to a positive selection agent that selects for cells expressing the selectable drug marker, thereby producing a selected population;

(d) culturing the selected population in the substantial absence of said agent and

(e) exposing the selected population to a negative selection agent which selects for cells which substantially lack expression of the selectable drug marker,

thereby generating a doubly-selected subpopulation enriched in position effect reporter cells wherein a reporter cell has an integrated a

reporter expression cassette comprising a nucleotide sequence exhibiting position effect.

26. A method for determining whether a test agent inhibits chromatin position effect comprising the steps of:

(a) providing a population of position effect reporter cells, each cell comprising an integrated reporter expression cassette that comprises a transcription regulatory region operably linked to a nucleotide sequence encoding a reporter polynucleotide, wherein expression of the nucleotide sequence in the population is greater in a cell population exposed to an agent known to inhibit position effect than in a population not exposed to the agent;

(b) culturing the population without an agent known to inhibit position effect;

(c) contacting the cell with the test agent; and

(d) detecting increased expression of expression of the reporter polynucleotide;

whereby detecting increased expression provides a determination that the test agent inhibits chromatin position effect.

27. The method of claim 26, wherein the transcription regulatory region comprises the hTR gene promoter region.

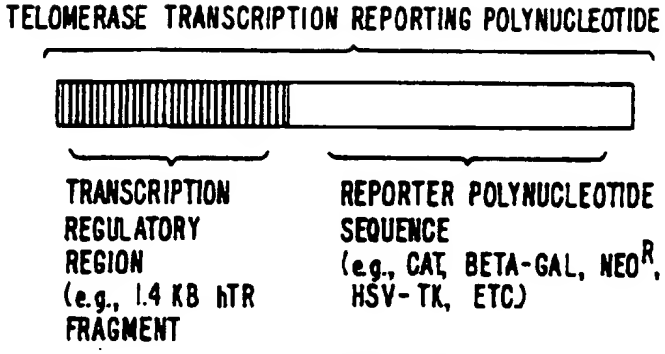
TELOMERASE TRANSCRIPTION REPORTING POLYNUCLEOTIDE
TRANSCRIPTION REGULATORY REGION (e.g., 1.4 KB hTR FRAGMENT
REPORTER POLYNUCLEOTIDE SEQUENCE (e.g., CAT, BETA-GAL, NEO^R, HSV-TK, ETC.)

FIG. 1A.

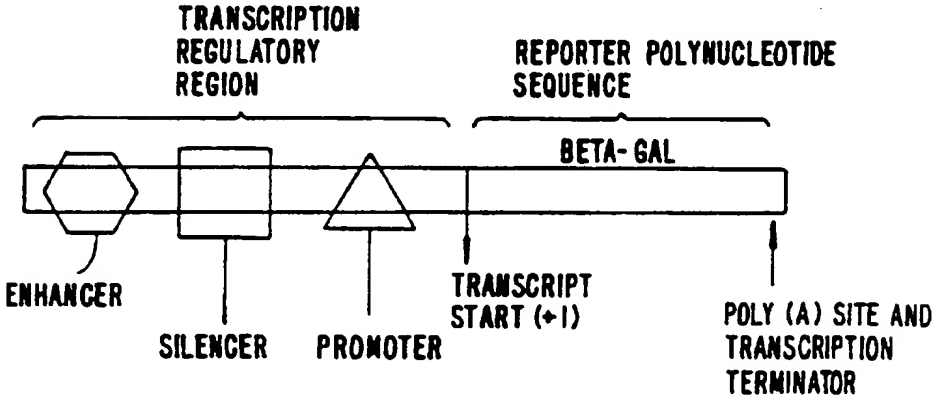
TRANSCRIPTION REGULATORY REGION
REPORTER POLYNUCLEOTIDE SEQUENCE
BETA-GAL
ENHANCER
SILENCER
PROMOTER
TRANSCRIPT START (+1)
POLY (A) SITE AND TRANSCRIPTION TERMINATOR

FIG. 1B.

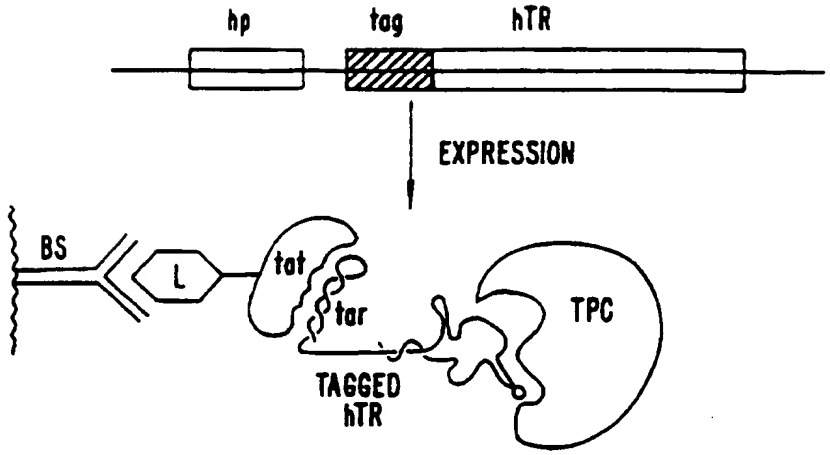

FIG. 2.

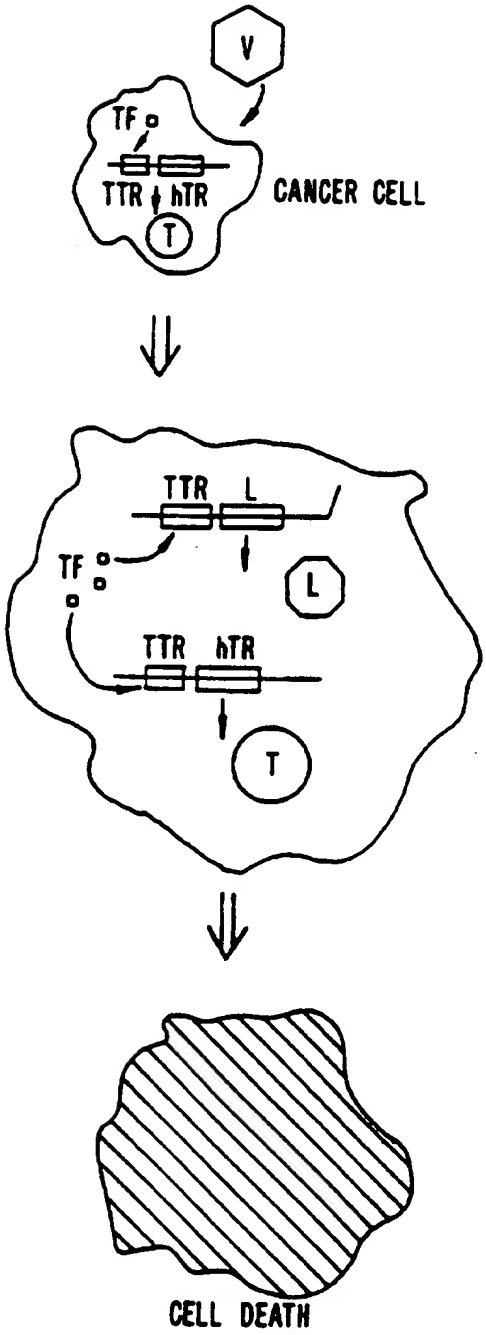

FIG. 3.